

NEET- 2020- 45 Days Crash Course



Date : 24th Aug 2020

Chapter Name : MOLECULAR BASIS OF INHERITANCE

Lecture Outline : REPLICATION TRANSCRIPTION TRANSLATION DNA FINGER PRINTING AND HUMAN GENOME PROJECT

A HISTORY OF DNA First identified - Fridrich Meischer 1869 - Milen

- Discovery of the DNA double helix (889)
 A. Frederick Griffith Discovers that a factor in diseased bacteria can transform harmless bacteria into deadly bacteria (1928)
 - B. Rosalind Franklin X-ray photo of DNA. (1952)
 - C. Watson and Crick described the DNA molecule from Franklin's X-ray. (1953)







Nucleotides <u>IMPINFO</u> Length : noz meledidest a pariz meledides (Benepaus) Barterighage \$X174 5386 milithides) Borten phage & - 48502BP. Humon - 6.6 y104 bp (3.37109 ún 6 Naplong) K. Coli - 4.6×10 B.P

Genetic Material - Introduction





Experiment on Transformation - 1

- When S- type bacteria injected into mice. The latter died as a result of pneumonia caused by bacteria.
- When R- type bacteria injected into mice. The latter lived and pneumonia was not produced.
- S- type bacteria which normally because disease was heat killed and then injected into the mice. The mice lived and pneumonia was not caused.
- The mixed solution of Rough type bacteria (living) and smooth type heat-killed bacteria (both known not to cause disease) injected into mice. Some mice died due to pneumonia and virulent smooth type living bacteria could also be recovered from their bodies.
 R Mpr LMN + Her Killed

The phenomenon is called **Griffith effect or transformation**. They proved that this substance is in fact DNA.

Purified biochemical from the killed S-type bacteria into three components - DNA, carbohydrate and protein.

DNA fraction was further divided into two parts: one with deoxyribonucleic or DNase and the other without it. The four components were then added to separate culture tubes containing R-type bacteria. After some time, they were then analysed for bacteria.

Experiment on Transformation - 2

DNA of S-type can have changed R-type of bacteria into S-type. Therefore, the character or gene of virulence is located in DNA. Thus they proved that the chemical to be inherited is DNA and it forms the chemical or molecular basis of heredity.



AVERY Mc Leod and McCarty Experiment

Heat kelled Strain Barlina DNA, RNA, Protei Mice Step I -DNA + RSTrain -> RNA + RSTran Ma Step II > Protein + Rstra Much SKPITT -Heart Kille SIVan - to silvan + Dhane -SterIV FRSTVart Knaser 11 Step V + RStvant / YO FC1 V

Transduction - Multiplication of Bacteriophage

The transfer of genetic material from one bacterium to another through bacteriophage is called **transduction**. $(\mathcal{U}_{3}) \sim \mathcal{U}_{3}$ Back \mathcal{U}_{3} (\mathcal{U}_{3})



Nucleic Acids

Chemical analysis of chromosomes shows presence of two nucleic acids- DNA (Deoxyribose nucleic acid) and RNA (Ribo nucleic acid).

Nucleic acid is a polymer of monomeric units, called **nucleotides**. Each nucleotide is composed of a **nucleoside and a phosphate group**. Thus nucleotide is a phosphoric ester of nucleoside.

- Nucleic acid = many nucleotides
- Nucleotide = nucleosides + phosphate
- Nucleoside = sugar + nitrogenous base
- Thus nucleotide = phosphate + sugar + nitrogenous base

Nucleotide monomer units join one another to give rise to polynucleotide chain.



Nucleotide Structure

RNA

1. Phosphoric acid: The acidic nature of nucleic acids is due to the presence of phosphoric acid Sugar of nucleoside combines with phosphoric acid by a phosphodiester bond formed at 5th or 3rd carbon of the sugar.

2. Sugar: It is a five carbon (pentose) sugar. There are two types of pentose sugars - **ribose and deoxyribose**. Deoxyribose sugar has one oxygen atom less at second carbon. Ribose sugar is present in RNA while deoxyribose sugar occurs in DNA.

D U A 3. Nitrogenous bases

Pyrimidine: It includes **cytosine**, **thymine and uracil**. Pyrimidine bases are made of only one ring of carbon.

1. (-

Purine: It includes **Adenine and guanine.** Purine bases are made of two ring of carbon and nitrogen bases of DNA contains adenine, guanine, cytosine and thymine. In RNA, uracil is present in place of thymine.

The two adjacent nucleotides are joined by formation of **phosphodiester bond** (a bond that involves two ester bonds).

A polynucleotide chain is often written as 5'p 3'OH, that it is a dinucleotide with phosphate group (p) attached to the 5th carbon of terminal nucleotide and hydroxyl group (OH) is present at 3rd carbon of basal nucleotide.

NUCLEOTIDE STRUCTURE



Pentose sugars



NITROGENOUS BASES



100

Structure of DNA (Deoxyribonucleic acid) - 1

J.D. Watson and F.H.C. Crick (1953) proposed double helical structure of DNA.



Structure of DNA (Deoxyribonucleic acid) - 2

Purine and pyrimidine base pairs are in equal amount, that is,

adenine + guanine = thymine + cytosine.

Ratio of A + T/G + C is constant for a species.

1. Each nucleotide consists of sugar, phosphate and a nitrogenous base. Many such nucleotides are linked by **phosphodiester bonds** to form a polynucleotide chain or strand.

2. Phosphodiester bonds are formed between 5' carbon of sugar of one nucleotide and 3' carbon of sugar of the next nucleotide.

3. Nitrogenous base is attached to 1' carbon of sugar. At this place purine base is attached by its 9' position and pyrimidine by its 3' position.

4. Polynucleotide strand is made of backbone of sugar and phosphate forming its long axis and bases at right angles.

Chargaff (1950) made observations on the base and other contents of DNA, called Chargaff's rule.

 $\begin{array}{c} A+G : & T+C \\ A=T, & G-C \end{array}$ DNAs the base ratio AT is always close to unity and the GC ratio also to always close to unity indicated that A always pairs with T and G pairs with C. A and T, G and C, therefore, ATT + atro is contain are complementary base pairs.

Pureno - Pynnudu

Structure of DNA (Deoxyribonucleic acid) - 3

One DNA strand has A, the other would have T and if it has G, the other, would have C. Therefore, if the base sequence of one strand is CAT TAG GAC, the base sequence of other strand would be GTA ATC CTG. Hence, the two poly **nucleotide strands** are called **complementary to one another**.

Such complementary strands are joined with one another by **hydrogen bonds** between their complementary nitrogenous bases. A = T, G = C

There are **three hydrogen bonds between cytosine and guanine** and **two hydrogen bonds between adenine and thymine** coiled around the same axis in such a way that can separate from one another only by uncoiling, is supposed to be right handed. Such a form of DNA is now called **B-DNA**.

Double stranded DNA molecule has a diameter of 20A°.

Helix makes one complete turn every 34 A^o along its length are 10 nucleotides per turn of - <u>34K</u> helix. Thus the distance between two neighbouring base pairs is 3.4 A^o.

Besides commonly known B-DNA, other forms are A, C (sometimes D and E) and Z DNA.

DNA Packing

In **prokaryotes e.g.** E. coli, though they do not have a defined nucleus, the DNA is not scattered throughout the cell. DNA (being negatively charged) is held with some Positively charged proteins in nucleoid region (an organised in large loops held by proteins.

In **Eukaryotes**, the DNA is folded with the help of positively charged basic proteins called **histones**.

Histones are low molecular weight, acting as repressor of genes to prevent transcription. These are rich in lysine and arginine. These are of 5 types (H1, H2a, H2b, H3, H4) depending upon the ratio of lysine, arginine and histidine.

Histones are organised to form a unit of eight molecules known as **histone octane**.

The negatively charged DNA is wrapped around the positively charged histone octamer to form a structure called **nucleosome or nu particle**.



2 nucleosomes called **linker DNA** (size 145 Å with 70 bp). **Nucleosome + DNA linker** is collectively called **chromatosome**.

H1 Protein helps in grouping of nucleosomes while nucleosomes help in packing of DNA. A chain of nucleosomes is once again coiled with 6 nucleosomes per turn to form **solenoid**

Properties of Genetic Material (DNA versus RNA)

It became an established fact that it is DNA that acts as genetic material. However, it subsequently became clear that in some viruses, **RNA is the genetic material (for example, Tobacco Mosaic viruses, QB bacteriophage).**

2'-OH group present at every nucleotide in **RNA** is a reactive group makes RNA labile and easily degradable, now known to be catalytic, hence reactive.

DNA chemically is less reactive and structurally more stable when compared to RNA, among the two nucleic acids, the DNA is a better genetic material. In the presence of thymine at the place of uracil also confers additional stability to DNA.

DNA and RNA are able to mutate, being unstable, mutate at a faster rate. Consequently, **viruses** having RNA genome and having shorter life span **mutate and evolve faster.**

DNA can function as genetic material, being more stable is preferred for storage of genetic information. **For the transmission of genetic information, RNA is better.**



Difference between DNA and RNA

S.No	DNA	RNA
1	It usually occurs inside nucleus and some cell	Very little RNA occurs inside nucleus.
	organelles.	Most of it is found in the cytoplasm.
2	DNA is the genetic material.	RNA is not the genetic material except in
		certain viruses, e.g. Reovirus.
3	It is double stranded with the exception $\rightarrow \pi$	RNA is single stranded except in certain viruses,
	of some viruses (e.g. psi × 174) - 5	double stranded Reovirus.
4	DNA contains over a million nucleotides.	Depending upon the type, RNA contains
		70-12000 nucleotides.
5	DNA is of only two types; intra-nuclear and	There are at least three types of RNAs– mRNA,
	extra-nuclear.	rRNA and tRNA.
6	It contains deoxyribose sugar.	It contains ribose sugar.
7	Nitrogen base thymine occurs in DNA alongwith	Thymine is replaced by uracil in RNA The other
	three others–adenine, cytosine and guanine.	three are similar – adenine, cytosine and
	A, G, TC	guanine. Treplaced by
8	It replicates to form new DNA molecules.	It cannot normally replicate itself. RNA
	DNA transcribes genetic information to RNA.	translates the transcribed message for
		forming polypeptides.
9	DNA control metabolism and genetics.	It only controls metabolism under
	including variation.	instruction from DNA.
10	Purine and pyrimdine bases are in equal number.	There is no proportionality between number of
		purine and pyrimidine bases. NO UNIN
		webuch

Meselson and Stahl Experiment - 1



SYNAMPSIJ

Found that DNA was intermediate type in first generation in which one strand was heavy (containing N_{15}) and other strand was light (containing N_{14}).

Meselson and Stahl Experiment - 1



Second generation of bacteria contained two types of DNA, 50% light (N¹⁴N¹⁴) and 50% intermediate (N¹⁵N¹⁴).

Third generation of bacteria contained 25% intermediate ($N^{15}N^{14}$) and 75% light in 1 : 3 ratio and fourth generation bacteria contained 12.5% $N^{15}N^{14}$ and 87.5% $N^{14}N^{14}$ DNA in 1 : 7 ratio.

Meselson and Stahl Experiment results





Mechanism of DNA Replication – Replication Fork



RNA Primer Small strand of RNA (5–10 nucleotide) synthesized at 5'end of new strand of enzyme Primase. Primer constitutes the initiation phase without the presence of RNA primer, DNA polymerase cannot add nucleotides.



Mechanism of DNA Replication - 2

In eukaryotes, the function of primase is carried out by enzyme DNA polymerase.

DNA Polymerase:



Prokaryotes possess three types of DNA synthesising enzymes called **polymerases III, II** and I they add $5' \rightarrow 3'$ direction on $3' \rightarrow 5'$ strand. DNA replication is mainly performed by DNA polymerase III. DNA polymerase I is major repair enzyme whereas polymerase II is minor repair enzyme.

In eukaryotes five types of DNA polymerases (a, b, c, d, e) have been reported. a, d, e are major enzymes.

Base Pairing: Separated strands of DNA in the replication fork function as template.

With the help of **pyrophosphatase enzyme** the two extra phosphates present on the Deoxyribonucleotides separate establishing hydrogen bonds between the free nucleotides and nitrogen bases of templates.

Chain formation

It requires DNA polymerase III in prokaryotes and polymerase in eukaryotes, Polymerase III is a complex enzyme having seven subunits

Replication on one DNA template is continuous in 5'-3' direction due to opening of newly formed strand is called **leading strand.**

Mechanism of DNA Replication - 3

In the presence of Mg++, ATP/ GTP, TPP and DNA polymerase -III, the nucleotides attached to nitrogen bases of each template DNA strand establish phosphodiester bonds and get linked to form replicated DNA strand antiparallel to each other.

Replication of DNA is <u>discontinuous</u> due to opening of small stretch of fork at a time. Small fragments deposited with the help of RNA these fragments are called **okazaki fragments**

Deposition of each okazaki fragment RNA primer is filled by the activity of DNA polymerase thus the new strand is called Lagging strand.

Deposition of bases DNA Ligase enzyme seals these bases.

One strand grows continuously while the other strand is formed discontinuously hence DNA replication is semi discontinuous.



Proof reading and DNA repair:

DNA polymerase I removes the wrong base and attaches the correct base in the strand in Prokaryotes whereas DNA polymerase b in eukaryotes.

Three Stages of replication 1). Initiation

- occurs at the origin of replication
- separates dsDNA, primer synthesis

2). <u>Elongation</u>

- involves the addition of new nucleotides
 (dNTPs) based on complementarity of the template strand
- forms phosphoester bonds, correct the mismatch bases, extending the DNA strand, ...

3). Termination

stops the DNA Replication occurs at a specific



Transcription - Introduction

A gene or cistron composed of stretch of Deoxyribonucleotides biochemical controlling other cistrons, rRNA, tRNA or polypeptide through mRNA performs one function, structural or regulatory.

Transcription:

Synthesis of RNA from DNA is called transcription.

Strands of DNA takes part in transcription. According to **Lewin** (2000)

TA N



Promoter is situated upstream of structural gene at 5' end of coding strand whereas terminator at downstream of structural gene at 3' end of coding strand.

Transcription Mechanism - 1

part 2 prit having conserved

Promoter bears parts for attachment to various transcription factors of the cases, the promoter contains AT rich regions called TATA box (Pribnow box). The latter has groove for the attachment of specific protein.

Structural gene is a part of strand of DNA having 3'-5' polarity on which transcription proceeds only in 5'-3' direction of new strand.

This strand of DNA is called template strand or master strand or antisense, or (–) strand.

The other strand is non – template strand that does not take part in transcription is also called **sense or coding strand or plus (+) strand.**

In G_1 and G_2 phases of interphase of cell cycle in the nucleus in eukaryotes where as in prokaryotes it occurs in the cytoplasm.



TRANSCRIPTION

K W A pol & subunit - Binds to pr motor B- DNA temp DNA helix Promoter (0) (N NO uburi) Sigma factor RNA polymerase Initiation enz **)** 22 Terminator RNA Elongation signe 1B' RNA Termination Polymerase RNA -Rho factor 1 Prokaro - only and RNA poly , with at Enkaryoti eing 2 Tomin RNAPHT - VRNA J > hn & m RNA M_ &RNA

Transcription Mechanism - 2

Single RNA in Prokaryotes. The (sigma) factor recognizes the site of transcription on the promoter region of DNA template and resting part of enzyme is called core enzyme.

Cistron: Prokaryotes bear polycistronic RNA and Eukaryotes bear monocistronic RNA.

Helicase enzyme performs uncoiling of DNA strands. SSB prevents their recoiling.

Sigma factor binds on TATA box on the promoter region of master strand. $R W A P d I_A A$

When the chain of RNA reaches at **terminator region**, **rho factor (r factor)** prevents its synthesis by ATPase activity resulting newly synthesized RNA becomes separated. Which is called **Primary transcript or Hn RNA (Heterogeneous RNA)**.

After separation of RNA, both strands of DNA are again coiled for duplex formation.

(i) Cleavage: Ribonuclease – P enzyme cleaves 5–7 bases of primary transcript may form t–RNA precursors.

(ii) Splicing: primary transcript, Introns have no information about protein synthesis.
 SnRNA (Small nuclear RNA) combines with some peptides to form small nuclear Ribonucleo protein or snurp. The latter combines with some peptides to form spliceosome, cut the introns of primary transcript and the exons of primary transcripts are joined by RNA ligase & thus active m–RNA is formed.

Transcription Mechanism - 3

(iii) Terminal addition: terminal part of Primary transcript for example – CCA sequence is added on 3' end of t-RNA, Poly A is added on 3' end of hn–RNA in tailing on the polyadenylation,

LARNA

In **capping**, **7– methyl guanosine** (formed by modification of GTP) is added on 5' end of hn–RNA.

(iv) Nucleotide modification: Nucleotides are methylated, ethylated or deaminated Ex. Inosine, methyl cytosine, Methyl guanosine, dihydrouracil, Pseudo uracil.

Represents relationship of sequence of Amino acids in polypeptide and sequences of nucleotides of mRNA/DNA.

(a) Triplet codon : Genetic code is Triplet codon composed of three adjacent nitrogen bases.

(b) Codon : A sequence of three nucleotides specifying an amino acid

- (c) Start signal or Initiation codon: It is mostly AUG (Methionine codor). It can be GUG and UUG, cases they specify Methionine. GUG and UUG specify different amino acids inside the polypeptide chain (GUG Valine, UUG- Leucine).
- (d) Stop signal or Termination codon: Polypeptide chain termination is signalled by three termination codon UAA (ochre), UAG (Amber) and UGA (opal). they do not specify any amino acid and hence are called non sense codons.

Post transcriptional modification



Wobble hypothesis, Central dogma and Reverse transcription

Wobble hypothesis (crick, 1966): first two nitrogen bases are similar while the third one is different. The third nitrogen base has no effect on coding actually 5' end base of t-RNA anticodon is able to wobble and get paired with even non-complementary base of m-RNA Ex: CCA CCC, CCG, and CCU III specify amino acid proline.

Central dogma: It is the unidirectional flow of information that proceeds from DNA to mRNA (**Transcription**) and then decoding information present in m-RNA in the formation of polypeptide chain or protein (**Translation**).



Concept of central dogma was proposed by crick in 1958.
 Circular flow of information (from DNA → RNA → Protein RNA → DNA)

Reverse transcription:

RNA

Retroviruses operate a central dogma reverse. RNA of these viruses first synthesizes DNA through **reverse transcriptase** or RNA dependent DNA polymerase. This DNA synthesized on RNA template is called **c-DNA or Retroposon**.

Protein synthesis (Translation)

Activation of Amino acid: combines with <u>t-RNA to</u> form Amino acyl t-RNA complex **(Charging or** Amino **acylation of tRNA).** That is also called charged t-RNA. Enzyme & AMP are released.



Synthesis of Polypeptide Chain

Initiation of polypeptide chain: Growing polypeptide chain **t**RNA **t**RNA CGTCAA CUCUUGGGUCCGCAGUUAA UUUCUAU mRNA Ribosome

Prokaryotes IF_3 , IF_2 , IF_1 , - Initiation factors are required for initiation of polypeptide chain, whereas in eukaryotes eIF_2 , eIF_3 , eIF_1 , eIF_{4A} , eIF_{4B} , eIF_{4C} , eIF_{4D} , eIf_5 , eIF_6 are required.

m-RNA is fused with P-site of small subunit 40S of ribosome (30 S in prokaryote), 40 S **mRNA complex** (30 S mRNA complex forms in the presence of IF_3).

40 S mRNA complex attracts **Amino acyl t-RNA Complex** both fuse to form 40 S m RNA + t-RNAfMet complex (30 S m RNA + t RNAfmet complex in prokaryotes in the presence of elF_3 and GTP.

Large subunit 60 S of ribosome (50 S) form 80 S mRNA t- RNA complex in the presence of eIF1, eIF4A, eIF4B, eIF4C, (70 s m RNA tRNA complex in the presence of IF1).

The 0.001M concentration. of Mg⁺⁺ ions are required for the formation of intact ribosome.

GENETIC CODE TABLE

SECOND LETTER

